The role of Tubulin β-III in cranial neural crest cell determination

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Introduction

Neural crest (NC) cells are a vertebrate-specific stem-like cell population that undergo an epithelial to mesenchymal transition (EMT) and then migrate to diverse locations within the developing embryo to create various derivatives. This migration takes place during and after neurulation, when the neural plate rolls into the neural tube. NC cells are formed at the dorsal region of this tissue. Premigratory NC cells are thought to be multipotent progenitor cells that can give rise to multiple cell type. NC cells can become neurons, melanocytes, cartilage, glia, and form the bulk of the enteric and peripheral nervous systems (Acloque et al., 2009). There is an established gene regulatory network that outlines the molecular pathways that drive the formation and differentiation of NC cells (Simões-Costa, 2015). However, there are still questions remaining about the timing of NC cell derivative differentiation. To understand the details of this process, our lab has characterized the spatiotemporal expression and function of Tubulin β-III (TUBB3), a protein that is an established neuronal marker (Lee et al., 1990). Here, we performed immunohistochemistry (IHC) and observed that TUBB3 is expressed prior to neurogenesis in the neural plate and in premigratory NC cells. Using antibodies marking definitive NC cells (SOX9 and SNAI2) we identified that TUBB3 co-localizes with NC markers prior to NC EMT. Additionally, there are populations of cells that are positive for TUBB3 and NC markers, but also cells that individually express each protein. We additionally identified that TUBB3 expression differs in the cranial and trunk regions. To determine the role of TUBB3 in early chick development, we used a translation-blocking TUBB3 Morpholino and performed subsequent IHC for markers of neural progenitors and NC cells. We identified that loss of TUBB3 expands SOX2+ neural tube progenitors into the dorsal neural tube at the expense of NC cells. We hypothesize that TUBB3 plays a role in NC specification as well as EMT. Future studies will identify these roles.

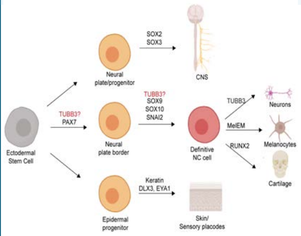


Figure 1. The traditional role of cell fate determination factors. Ectodermal stem cells become neural progenitors, epidermal progenitors, and neural plate border cells, which express Pax7 and become NC cells (Murdoch et al., 2012). For these cells to become definitive NC, Sox9 or Snai2 must be activated (Stuhlmiller et al., 2012). Definitive NC cells then activate genes that will drive the cells them towards the pathway of a specific derivative. Questions still remain about ***when*** NC cells know what they will become.

Results

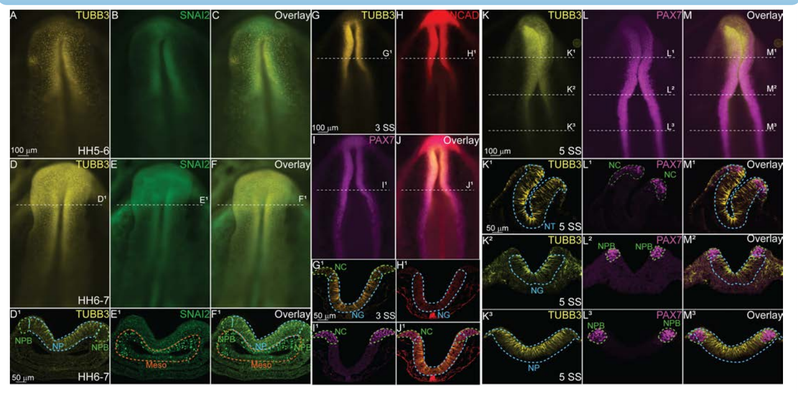


Figure 2. TUBB3 is expressed in the developing neural plate prior to NC specification. Immunohistochemistry (IHC) using antibodies against TUBB3, a neuron marker (yellow), SNAI2, a definitive NC marker (green), N-cadherin (NCAD), a cell adhesion molecule (red), and PAX7, a neural crest progenitor marker (magenta). At stage HH5-6 and stage 6-7 TUBB3 is expressed by cells in the neural plate (NP) and neural plate border (NPB) prior to the neural tube closure. (A-F, G-J, K-M) are whole mounts with anterior to the top and posterior to the bottom while (D^1-F^1, G^1-J^1, K^1-M^3) are transverse sections with dorsal to the top and ventral to the bottom. These data suggest that TUBB3 protein may have early roles in addition to later fate determination. Cranial mesenchyme/mesoderm is Meso. Scale bars are as marked.

Results

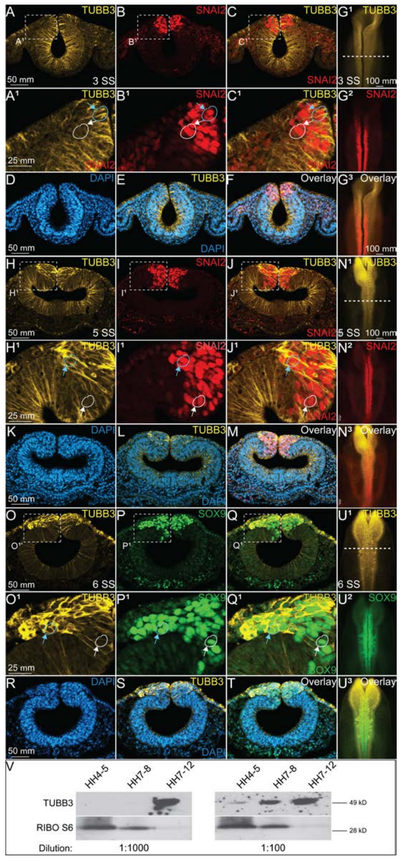


Figure 4. TUBB3 is maintained in cranial ganglia. IHC using antibodies against TUBB3 (yellow), SOX9 (green), PAX6 (red), and SOX2 (magenta) and stain for DAPI (blue) in multiple stages of chick embryos. (A^1-D^3) Transverse sections at different axial levels from 15 SS embryo indicated in (A-D) show that TUBB3 co-localizes with SOX9+ migratory cranial NC cells. (E^1-H^1) Coronal section of 20 SS embryo indicated in (E-H) shows that TUBB3 maintains expression in the brain and developing eye. (I^1-L^1) Transverse sections from 21 SS embryo indicated in (I-L) show that TUBB3 co-localizes with SOX9+ cells that are condensing to form the TG. (M-P) Verification that TUBB3 is expressed in differentiating cranial neurons. (R= retina, L= lens, NC= neural crest, Di= diencephalon, TG= trigeminal ganglia, OV= olfactory vesicle, TN= trigeminal nerve, ON= optic nerve, OpthB= ophthalmic branch. Scale bars are as marked.

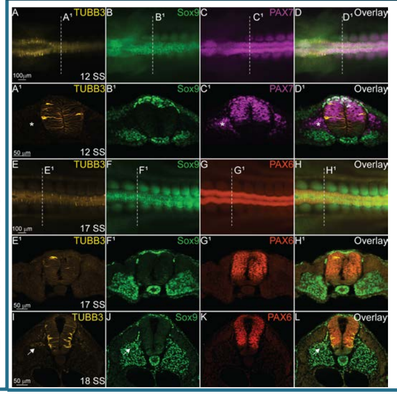


Figure 5. TUBB3 does not co-localize with trunk NC cells. IHC using antibodies against TUBB3 (yellow), SOX9 (green), PAX7 (magenta), and PAX6 (red) in the spinal cord of various staged embryos. TUBB3 is expressed intermittently in the developing spinal cord (A^1, E^1) at 12 SS and 17 SS, but expression becomes more stereotypic of differentiating spinal neurons at 18 SS (I). (A-D, E-H) Whole mount embryos with anterior to the left and posterior to the right. Dorsal side is facing reader. (A^1-D^1, E^1-H^1, I-L) Transverse sections with dorsal to the top and ventral to the bottom. Asterisks indicate TUBB3-/PAX7+ cells and arrows indicate TUBB3+/SOX9+ cells. Scale bars are as marked.

Results

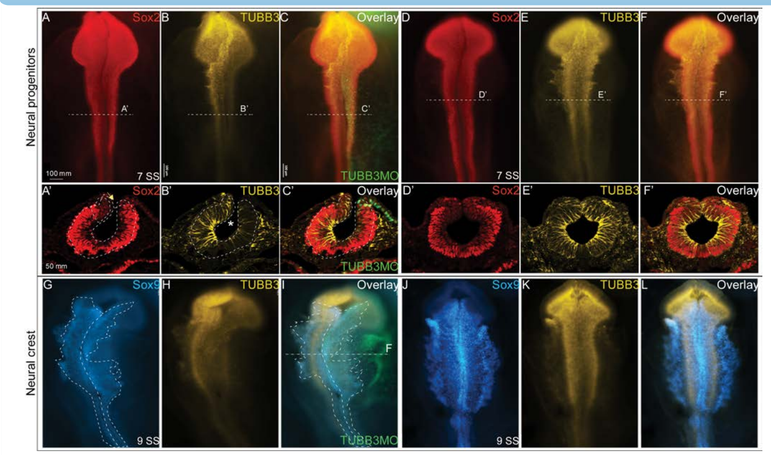
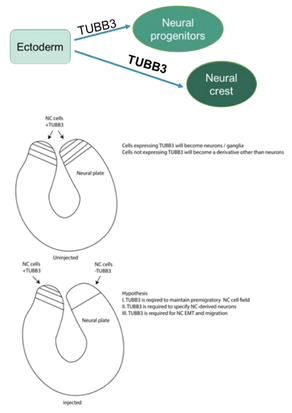


Figure 6. Loss of TUBB3 creates neuroectodermal defects. IHC for (A, D) SOX2 (red), (B, E, H, K) TUBB3 (yellow), and (G, J) SOX9 (blue) was performed on embryos injected with translation-blocking TUBB3 morpholino (TUBB3MO, green) or uninjected wildtype embryos. Embryos were injected and electroporated at gastrula stage and collected at 7 SS (SOX2) or 9 SS (SOX9) to assess the effects on neural progenitors and NC cells, respectively. (A-C) SOX2+ cells are expanded into the dorsal neural tube after TUBB3 knockdown compared to uninjected side and (D-F) wildtype embryos. (G-I) SOX9 expression is abnormal after TUBB3 knockdown compared to (J-L) wildtype embryos.

Summary



- ❖TUBB3 is expressed in the developing neural plate during neurulation prior to definitive NC cell markers.
- ❖TUBB3 expression increases in the dorsal neural tube during NC specification, prior to EMT.
- ❖Late stage embryos confirm TUBB3 expression in ganglia but does not co-localize with trunk NC cells.
- ❖Loss of TUBB3 may lead to neuroectodermal patterning defects, expanding SOX2 and reducing SOX9.

Future Directions

- Conduct additional gain and loss of function experiments.
- Perform additional comparative IHC using markers of NC derivatives to see if TUBB3 is neuron specific or if it co-expresses with other markers.
- Determine if alterations in TUBB3 expression change proportions of NC derivatives (neurons vs. melanocytes vs. cartilage) at later stages.

References

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Acknowledgements

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